

REMARKS

The Office action mailed 28 December 2010, has been received and its contents carefully noted. Claims 29-36, 39 and 40 were pending, claims 31-34 and 36 were withdrawn from consideration, and claims 29, 30, 35, 39-42 were rejected. By this Response, claims 29 and 42 have been amended and claim 43 is newly added. Support may be found in the Specification and claims as originally filed. No statutory new matter has been added. Reconsideration in view of the following are respectfully requested.

Rejection under 35 U.S.C. 112, second paragraph

The Examiner rejected claim 42 under 35 U.S.C. 112, second paragraph, as being indefinite. Specifically, the Examiner deemed that the term "cartridge" is not defined by the claim or the specification by the number of substrates included. The Examiner also deemed that the use of "comprising" makes it unclear what components are in the cartridge.

Applicants respectfully submit that the claims as amended are clear and definite. Specifically, the paragraph bridging pages 28 and 29 of this specification make clear that the cartridge may comprise all the reagents, i.e. all the substrates. Nothing more is needed. The simple fact that the cartridge may comprise additional reagents does not make the claims indefinite. To hold such based on the term "comprises" is equivalent to saying that all patent claims which use "comprises" as a transitional phrase are indefinite as the claims do not explicitly set forth all the additional features which may be included. Further, Applicants respectfully submit that what may be "unlikely" has no impact on whether a claim is indefinite. The claims, as amended, make clear that the cartridge must contain at least two substrates. The fact that the cartridge may contain additional substrates does not make the claims indefinite.

Therefore, the rejection under 35 U.S.C. 112, second paragraph, should properly be withdrawn.

Rejection under 35 U.S.C. 102(b)

The Examiner rejected claims 29, 30, 35, 39 and 40 under 35 U.S.C. 102(b) as being

anticipated by London (1995). Specifically, the Examiner found Applicants' prior arguments unpersuasive as the Examiner deemed that London discloses the Test-Mate OP kit which is deemed to comprise two substrates, i.e. butyrylthiocholine and acetylthiocholine.

Applicants respectfully submit that the Examiner mischaracterizes the disclosure of London and/or does not appreciate the contents of the Test-Mate OP kit. Specifically, one Test-Mate OP kit (either the PCE field kit or the ECE field kit) do not contain two substrates, i.e. butyrylthiocholine and acetylthiocholine. Instead, the PCE field kit contains butyrylthiocholine, but does not contain acetylthiocholine. Similarly, the ECE field kit contains acetylthiocholine, but does not contain butyrylthiocholine. This is evidenced by the package insert for the AChE Assay Kit and the PChE Assay Kit available from EQM Research, Inc. (the commercial vendor of the Test-Mate OP Kits) (enclosed). See the Contents and Reagents sections as follows:

Contents

Each assay kit contains three boxes and a package insert. Box one contains 48 assay buffer tubes. Box two contains 48 assay buffer tubes. Box three contains a 96 well reagent plate, 100 capillary tubes (10µL volume), 100 filter papers (capillary wipes), a clear plastic dropper bottle filled with 18mL of reagent solvent, 2 transfer pipettes and a 9 Volt battery. The reagent plate in the AChE Assay Kit has a red "Erythrocyte" label and the PChE Assay Kit has a blue "Plasma" label. The transfer pipettes in the AChE Assay Kit have a red band and the transfer pipettes in the PChE Assay Kit have a blue band. *Note: Never interchange the reagent plate or the transfer pipettes when switching between AChE and PChE testing.*

- Erythrocyte Cholinesterase Reagent: (AChE Erythrocyte Cholinesterase Assay Kit)**
Lyophilized, 96 tests per plate. Store lyophilized reagent at 15 - 30°C, protected from light. Reconstitute with 3 drops of reagent solvent. Stable 72 hours at 15 - 30°C after reconstitution. Final assay includes: 1mM AcTC, 0.3mM DTNB, 20µM As1397, 50mM potassium phosphate and 0.03% Triton X-100 (white cap), pH 7.6.
Plasma Cholinesterase Reagent: (PChE Plasma Cholinesterase Assay Kit)
Lyophilized, 96 tests per plate. Store lyophilized reagent at 15 - 30°C, protected from light. Reconstitute with 3 drops of reagent solvent. Stable 72 hours at 15 - 30°C after reconstitution. Final assay includes: 2mM BuTC, 0.3mM DTNB, 50mM potassium phosphate and 0.03% Triton X-100 (white cap), pH 7.6.

The package insert makes clear that AcTC is acetylthiocholine and the BuTC is butyrylthiocholine. The Test-Mate OP kit for AChE (ECE) contains the erythrocyte cholinesterase reagent (which only contains AcTC) and the Test-Mate OP kit for PChE contains plasma cholinesterase reagent (which only contains BuTC). The package insert specifically teaches against interchanging the reagent plate or the transfer pipettes when switching between AChE and PChE testing (so as to prevent contamination).

Nowhere does London teach or suggest using both acetylthiocholine and butyrylthiocholine to measure BChE. Nowhere does London teach or suggest using both acetylthiocholine and butyrylthiocholine to measure AChE. Nowhere does London teach or suggest a device which comprises both acetylthiocholine and butyrylthiocholine.

According to the present invention as claimed, the device comprises a cartridge which contains $n+1$ number of substrates, where n is the number of proteins in the plurality of proteins and wherein n is a positive integer (support for this "positive integer" limitation is inherently supported in the instant specification in view of the fact that "at least one protein" is to be assayed, which thereby provides that n is at least one). Thus, the cartridge as set forth in the claims contains at least two substrates, i.e. one additional substrate to the number of proteins to be contacted with aliquots of the same sample in order to measure the reaction rates for each protein for each substrate as exemplified by the three equation sets in Example 2 of the specification. See page 32, line 27, to page 33, line 15. Although the proteins to be assayed are not structural limitations, the at least two substrates, i.e. $n+1$ substrates, limitation is an actual structural limitation which can not be disregarded as an intended use limitation.

Nowhere does London teach or suggest one device which has a cartridge containing at least two substrates. Nowhere does London teach or suggest accounting for the activity of a second protein in a test sample which overlaps with the activity of the protein of interest by using sensitivity coefficients according to the instant invention. Instead, London teaches using inhibitor As1397 to prevent PCE (i.e. BChE activity) interference when measuring AChE. Thus, based on the teachings of London, one skilled in the art would not have been motivated to (a) provide a cartridge contains acetylthiocholine and butyrylthiocholine in order to measure the activities or concentrations of one cholinesterase, i.e. AChE or BChE, or (b) a cartridge which contains acetylthiocholine, butyrylthiocholine, and one more substrate in order to measure the activities or concentrations of both AChE and BChE in aliquots of the same sample.

Consequently, London does not teach or suggest the claimed invention and the rejection under 35 U.S.C. 102(b) should properly be withdrawn.

Rejection under 35 U.S.C. 103(a)

The Examiner rejected claims 29 and 40 under 35 U.S.C. 103(a) as being unpatentable over London in view of Jacobs (1993).

Jacobs does not alleviate the deficiencies of London. Nowhere does Jacobs teach or suggest a device which contains at least two substrates and/or a device which contains one cartridge having at least two substrates.

Therefore, Applicants respectfully submit that the claimed invention is unobvious and the rejection under 35 U.S.C. 103(a) must properly be withdrawn.

Request for Rejoinder

Applicants respectfully request rejoinder of the withdrawn claims which ultimately depend on claim 29.

Request for Interview

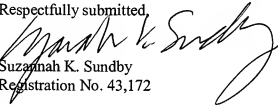
Either a telephonic or an in-person interview is respectfully requested should there be any remaining issues.

CONCLUSION

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Therefore, it is respectfully requested that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. It is believed that a full and complete response has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. However, in the event that additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. 1.136(a), and any fees required therefor are hereby authorized to be charged to **Deposit Account No. 210-380**, Attorney Docket No. **034047.003DIV1 (WRAIR 00-23)**.

Respectfully submitted,



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AChE Erythrocyte Cholinesterase Assay Kit (Model 460)
&
PChE Plasma Cholinesterase Assay Kit (Model 470)

Package Insert

Intended Use

For the quantitative determination of cholinesterase in whole blood to monitor pesticide exposure. For in vitro diagnostic use. For laboratory use by trained laboratory technicians only. The Test-mate ChE Cholinesterase Test System is useful in the assessment of pesticide poisoning. Most organophosphate or carbamate pesticides inhibit the blood enzymes erythrocyte cholinesterase (AChE) and/or plasma cholinesterase (PChE).^{1,2} The degree of enzyme inhibition is proportional to the extent of exposure. AChE is generally preferred because of its lower biological variability and lack of interferences relative to PChE. After exposure to pesticides, recovery of AChE activity is usually slower than PChE due to its longer half-life (1 month for AChE vs. 2 weeks for PChE).^{3,4} Pre-exposure (baseline) measurements of AChE and/or PChE should be obtained to reduce the effect of biological variability.¹

Principle of the Method

The Test-mate ChE reagents are based on the Ellman method.⁵ Acetylthiocholine (AcTC) or butyrylthiocholine (BuTC) is hydrolyzed by AChE or PChE, respectively, producing carboxylic acid and thiocholine which reacts with the Ellman reagent (DTNB, dithionitrobenzoic acid) to form a yellow color which is measured spectrophotometrically at 450nm. The rate of color formation is proportional to the amount of either AChE or PChE.



The AChE reagent is >95% specific due to the addition of a specific inhibitor of PChE, As1397 (10-(α -diethylaminopropionyl)-phenothiazine).⁶ BuTC is >95% specific for PChE.

Contents

Each assay kit contains three boxes and a package insert. Box one contains 48 assay buffer tubes. Box two contains 48 assay buffer tubes. Box three contains a 96 well reagent plate, 100 capillary tubes (10 μ L volume), 100 filter papers (capillary wipes), a clear plastic dropper bottle filled with 18mL of reagent solvent, 2 transfer pipettes and a 9 Volt battery. The reagent plate in the AChE Assay Kit has a red "Erythrocyte" label and the PChE Assay Kit has a blue "Plasma" label. The transfer pipettes in the AChE Assay Kit have a red band and the transfer pipettes in the PChE Assay Kit have a blue band. *Note: Never interchange the reagent plate or the transfer pipettes when switching between AChE and PChE testing.*

Instrumentation

The AChE Erythrocyte Cholinesterase Assay Kit and the PChE Plasma Cholinesterase Assay Kit are for use only with the photometric analyzer supplied as part of the Test-mate ChE Cholinesterase Test System and are not intended for use with any other manual or automated test method or equipment.

Reagents

1. **Buffer:** 2mL per assay tube. Contains phosphate, surfactant and EDTA preservative.
2. **Reagent Solvent:** 18mL of distilled water and EDTA preservative in a plastic dropper bottle.
3. **Erythrocyte Cholinesterase Reagent:** (AChE Erythrocyte Cholinesterase Assay Kit)
Lyophilized, 96 tests per plate. Store lyophilized reagent at 15 - 30°C, protected from light. Reconstitute with 3 drops of reagent solvent. Stable 72 hours at 15 - 30°C after reconstitution. Final assay includes: 1mM AcTC, 0.3mM DTNB, 20µM As1397, 50mM potassium phosphate and 0.03% Triton X-100 (white cap), pH 7.6.
4. **Plasma Cholinesterase Reagent:** (FChE Plasma Cholinesterase Assay Kit)
Lyophilized, 96 tests per plate. Store lyophilized reagent at 15 - 30°C, protected from light. Reconstitute with 3 drops of reagent solvent. Stable 72 hours at 15 - 30°C after reconstitution. Final assay includes: 2mM BuTC, 0.3mM DTNB, 50mM potassium phosphate and 0.03% Triton X-100 (white cap), pH 7.6.

Specimen Collection

Either fresh fingerstick blood or venipuncture blood (anticoagulated with EDTA) can be used. The puncture site should be thoroughly washed before sampling, in order to minimize possible sample contamination from pesticide residue adsorbed to the skin. To avoid clotting, the capillary should be placed into the assay tube within 10 seconds. Cholinesterase can reactivate, especially from carbamate pesticide inhibition during prolonged storage. Such reactivation can produce a "false negative".¹

Test Procedure

1. Turn on the photometric analyzer. Press the **MODE** key to select either the AChE assay procedure or the FChE assay procedure. Press the **TEST** key to begin the assay.
2. Insert the new assay tube into the analyzer. Press the **TEST** key to continue the assay.
3. When prompted by the analyzer, remove the assay tube. Press the **TEST** key to continue the assay.
4. Fill the 10µL capillary with blood (wipe excess with filter paper) and place it into the assay tube. Vigorously shake the assay tube for 15 seconds. Align the capillary and then insert the assay tube into the analyzer. Press the **TEST** key to continue the assay.
5. When prompted by the analyzer, remove the assay tube. Press the **TEST** key to continue the assay.
6. Dissolve the reagent with 3 drops of reagent solvent. Add the dissolved reagent to the assay tube using the transfer pipette. Immediately, press the **TEST** key to continue the assay.
7. Gently shake the assay tube by inversion for 5 seconds. Align the capillary and then insert the assay tube into the analyzer. Press the **TEST** key to continue the assay.
8. When prompted by analyzer, remove and discard the assay tube. Press the **TEST** key to continue the assay.
9. Record the analyzer readings, using the **TEST** key to advance the display. Press the **DONE** key to finish the assay.

Calibration

The Test-mate ChE photometric analyzer is factory-calibrated. No additional calibration is required.

Quality Control

The use of an unexposed operator is best; the intraindividual variability of both erythrocyte and plasma cholinesterase is less than 5% per week and less than 10% per month.⁴ Alternatively, refrigerated venipuncture blood (anticoagulated with EDTA) is stable for at least one month. Controls should be run on each day of testing.

Calculations

The measured cholinesterase activity is calculated by the photometric analyzer using the following equation:

$$\text{U/mL blood} = \frac{(A/\text{min}) (\text{mL assay volume})}{(e, \text{mM}^{-1}\text{cm}^{-1}) (\text{cm light path}) (\text{mL blood})}$$

The measured cholinesterase activity is further refined by the following adjustments to derive the final displayed cholinesterase value:

Reagent Blank Adjustment: A small (approximately 15%) nonspecific blank reaction is subtracted from the measured cholinesterase activity.

Temperature Adjustment: Using the temperature sensor in the analyzer, both the measured cholinesterase activity and the reagent blank activity are normalized to 25°C.

Hemoglobin Adjustment: For AChE, hemoglobin normalizes varying sample size and iron status; therefore AChE is most accurately expressed as U/g Hgb.

Limitations

Physiological Interference: AChE is depressed in paroxysmal nocturnal hemoglobinuria (PNH).⁴ In severe macrocytic or microcytic anemia, the ratio of hemoglobin/cholinesterase may interfere with hemoglobin correction and therefore, AChE activity. PChE is depressed in liver failure and malnutrition. PChE is increased in alcoholic/viral hepatitis and infection.⁵

Analytical Interference: Drugs which inhibit cholinesterase, such as pyridostigmine, will decrease cholinesterase. Pesticide residues adsorbed to the skin can artificially decrease values.⁶ Washing the skin with quaternary ammonium-containing detergents, such as benzethonium chloride can also artificially decrease values; check the detergent label before using.

Accuracy

The Test-mate ChE was compared with the Boehringer Mannheim Cholinesterase Kit No. 450035 on the Hitachi 704 (BM/Hi).¹⁰ The BM/Hi method is performed on plasma (PChE) or diluted whole blood (AChE) corrected by hematocrit; therefore, in contrast to the Test-mate ChE units of U/mL whole blood at 25°C or U/g Hgb at 25°C, the BM/Hi results are expressed as U/L plasma (PChE) at 37°C or U/L RBCs (AChE) at 37°C.

Normal Donors: (X, BM/Hi, Venipuncture) vs. (Y, Test-mate, Fingertick)

| | N | r | Slope | Intercept | R _{range} [†] |
|--------------------------------|----|------|----------|-----------|---------------------------------|
| AChE, U/L RBCs vs. U/g Hgb | 44 | 0.78 | 0.000894 | 10.8 | ±25%CV |
| PChE, U/L plasma vs. U/L blood | 44 | 0.96 | 0.255 | 440 | ±50%CV |

Pesticide-Dosed & Normal Donors: (X, BM/Hi, Venipuncture) vs. (Y, Test-mate, Venipuncture)

| | N | r | Slope | Intercept | R _{range} [†] |
|---------------------------------|----|------|---------|-----------|---------------------------------|
| AChE, U/L RBCs vs. U/g Hgb | 86 | 0.98 | 0.00158 | 0.322 | ±100%CV |
| PChE, U/L plasma vs. U/L plasma | 87 | 0.98 | 0.457 | -210 | ±100%CV |

[†]Note: r, the correlation coefficient, is extremely range-sensitive (increases with %CV range).

Precision

Within-run, N=40, 1 - 5 U/mL: 3 - 5%CV.

Between-run, N=40, 1 - 5 U/mL: 5 - 7%CV.

Linearity

Erythrocyte AChE: 0 - 7 U/mL; 0 - 50 U/g Hgb. Plasma PChE: 0 - 7 U/mL.

Expected Values

These were determined using normal male and female blood bank donors, between 20 and 60 years of age, located in the Midwestern United States.

| | N | Mean | SD | Range |
|---------------|----|------|------|-------------|
| ACHE, U/mL | 40 | 3.68 | .47 | 2.77 - 5.57 |
| ACHE, U/g Hgb | 40 | 27.1 | 2.9 | 21.9 - 37.3 |
| PChE, U/mL | 40 | 2.03 | 0.40 | 1.35 - 3.23 |

Between-Operator Variability

Ten operators each performed ten measurements on both a normal and an abnormal venipuncture sample (N=100). The abnormal sample was prepared by dosing with pesticide (parathion).

| | Normal | | | | Abnormal | | | |
|------|--------------|-------------|--------------|-------------|--------------|-------------|--------------|-------------|
| | ACHE U/mL | ACHE U/g | PChE U/mL | Hgb g/dL | ACHE U/mL | ACHE U/g | PChE U/mL | Hgb g/dL |
| Mean | 5.63 | 33.8 | 1.72 | 16.8 | 1.38 | 9.7 | 1.03 | 14.3 |
| SD | 0.21 | 0.8 | 0.15 | 0.5 | 0.12 | 0.8 | 0.08 | 0.3 |
| %CV | 3.7 | 2.4 | 8.5 | 2.7 | 9.0 | 7.9 | 7.5 | 2.2 |

Interpretation of Results

Depression of cholinesterase to <50% normal indicates possible pesticide poisoning requiring removal from exposure and/or treatment with anticholinergics such as atropine and pralidoxime.¹ Suspected cases of poisoning can be confirmed by cholinesterase monitoring for a subsequent rise and plateau of activity 1 - 3 months after exposure. If baseline values are obtained, depression of cholinesterase to <70% of baseline can be taken to indicate possible pesticide poisoning.¹¹

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